DATA EVALUATION RECORD

PICOXYSTROBIN (ZA1963)

Study Type: OPPTS 870.4100b [§83-1b], Chronic Toxicity Study in Dogs

Work Assignment No. 7-1-256 G (MRID 48073741)

Prepared for
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This Data Evaluation Record my have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

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DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity in Dogs (diet); OPPTS 870.4100b [§83-1b]; OECD 452.

PC CODE: 129200 **DP BARCODE:** D378236
TXR #: 0056696 **SUBMISSION #:** S873059

TEST MATERIAL (PURITY): Picoxystrobin (94.4% a.i.)

SYNONYMS: ZA1963; methyl (α*E*)-α-(methoxymethylene)-2-[[[6-(trifluoromethyl)-2-pyridinyl]oxy]methyl]benzeneacetate

CITATION: Lees, D. (1999) ZA1963: 1 year dietary toxicity study in dogs. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Report No.: CTL/P/6049, January 18, 1999. MRID 48073741. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In a chronic toxicity study in dogs (MRID 48073741), picoxystrobin (ZA1963; 94.4% a.i., Batch No. P27) was administered in the diet to 4 beagle dogs/sex/dose for 1 year at doses of 0, 50, 150, or 500 ppm (equivalent to 0/0, 1.6/1.6, 4.8/4.6, and 16.1/15.7 mg/kg/day in males/females).

No adverse, treatment-related effects were observed on mortality, ophthalmology, hematology, clinical chemistry, urinalysis, organ weights, or gross or histological pathology.

At 500 ppm, females were considered thin (3/4 dogs, 62 total observations) compared to controls (1/4 dog, 11 total observations). Body weights were decreased by 5-11% in the males during Weeks 2-30. Body weights were decreased by 3-15% in the females throughout the study, with the exception of the final week. Males lost weight during the first 2 weeks, and females lost weight during the first week. Body weight gains for the interval of Weeks 1-13 were decreased by 75-77% in both sexes, contributing to overall (Weeks 1-53) decreased body weight gains of 27% in the males and 38% in the females. Generally throughout the study, food consumption decreased by 5-34% in males and by 6-36% in females.

The LOAEL is 500 ppm (equivalent to 16.1/15.7 mg/kg/day in males/females), based on decreased body weights, body weight gains, and food consumption in both sexes. The NOAEL is 150 ppm (equivalent to 4.8/4.6 mg/kg/day in males/females).

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.4100b, OECD 452) for a chronic oral toxicity study in dogs.

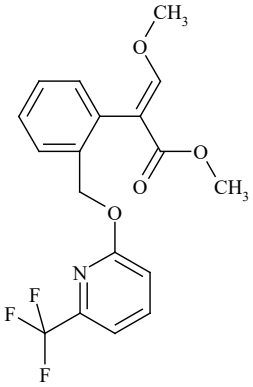
COMPLIANCE – Signed and dated GLP Compliance, Quality Assurance, and Data Confidentiality statements were provided. No Flagging Statement was provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Picoxystrobin

Description: Pale yellow solid
Batch No.: P27
Purity (w/w): 94.4% a.i.
Stability of compound: The test compound was stable in the dietary formulations for at least 42 days at room temperature.
CAS #: 117428-22-5
Structure:



2. Vehicle: Diet

3. Test animals

Species: Dog
Strain: Beagle
Age and group mean weight at study initiation: Approximately 20-29 weeks old; 8.95-9.25 kg males; 7.75-7.88 kg females
Source: Conventional Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, England
Housing: 4 per indoor pen (same sex and dosing group); dogs received exercise
Diet: Laboratory Diet A (Special Diets Services Limited, Stepfield, Witham, Essex, UK); 350 g for males and 300 g for females were supplied for 4 hours each day
Water: Tap water, *ad libitum*
Environmental conditions
- **Temperature:** 19±4ºC
- **Humidity:** 35-75%
- **Air changes:** Approximately 15/hour
- **Photoperiod:** 12 Hours light/12 hours dark

Acclimation period: Approximately 4-5 weeks

B. STUDY DESIGN

1. In life dates: Start: 8/12/1997 End: Approximately 8/12/1998

2. **Animal assignment:** The dogs were randomly assigned, stratified by body weight, to the following groups, while avoiding inclusion of litter mates within the same group (Table 1).

TABLE 1: Study design [a]

Nominal dose (ppm)	Actual dose (mg/kg/day)	Dogs/sex/dose
0	0/0	4
50	1.6/1.6	4
150	4.8/4.6	4
500	16.1/15.7	4

a Data were obtained from pages 19 and 24 of MRID 48073741.

3. **Dose-selection rationale:** The dose-selection was based on a previously conducted subchronic study in dogs. In a concurrently submitted subchronic oral toxicity study (MRID 48073734), ZA1963 was administered in the diet to four beagle dogs/sex/dose group at concentrations of 0, 125, 250, or 500 ppm for at least 90 days. In the 250 ppm males, body weights were decreased by 2-5% during Weeks 3-14, contributing to a 21% decrease in overall (Weeks 1-14) body weight gains. At 500 ppm, body weights were decreased during Weeks 2-14 by 4-7% in the males and by 3-7% in the females, contributing to overall decreased body weight gains of 38% and 45% in males and females, respectively. Additionally at this dose, food consumption was decreased during Weeks 1-6 by 7-27% in the males and by 8-26% in the females.

4. **Diet preparation and analysis:** The test compound was mixed with the diet to form a pre-mix, which was further diluted with appropriate amounts of the diet to achieve the desired concentrations. Water was added, the diet was made into pellets, and the pellets were dried. The frequency of preparation was not reported. Test compound stability in the diet was evaluated in the 50 and 500 ppm dietary formulations for up to 42 days at (assumed by the reviewer) room temperature. Homogeneity (top, middle, and bottom strata) was also evaluated in the 50 and 500 ppm dietary formulations. Concentrations were measured in 7 batches for the 150 ppm dietary formulation and 6 batches for the other concentrations during the study.

Results

Homogeneity (% coefficient of variation): 1.33% for 50 ppm and 1.79% for 500 ppm

Stability (% of initial): 98.4-105.3% (3 weeks after preparation)

Concentration (% of nominal): 80.7-105%

Dose (ppm)	Range (% of nominal)
50	90.8-96.2
150	80.7-98.7
500	90.2-105

With the exception of one batch of 150 ppm diet, the dietary formulations were within 10% of nominal. The outlier was within 20% of nominal and was replaced after 6 days. Therefore, the analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** All data were evaluated using the GLM procedure in SAS, separately for males and females. The following statistical analyses were performed.

PARAMETER	STATISTICAL ANALYSES
Body weight	Body weights were considered by analysis of covariance on initial (week 1) body weight. [a]
Food consumption	Analysis of variance was conducted. [a]
Hematology Clinical chemistry Urinalysis (quantitative)	Analysis of covariance on pre-experimental values was conducted. Male and female data were analyzed together and the results examined to determine whether any differences between control and treated groups were consistent between sexes. [a]
Organ weights	Analysis of variance and analysis of covariance on final body weight were performed. The data from paired organs were examined for differential effects on left and right components. [a]

a Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

These statistical analyses were considered appropriate.

C. METHODS

1. **Observations:** Animals were inspected at least twice daily for mortality, moribundity, and clinical behavioral abnormalities. Detailed weekly physical examinations were performed on each animal beginning 2 weeks prior to dosing. Gastro-intestinal findings were assessed daily for at least 2 weeks prior to dosing and throughout the treatment period. All dogs were also given a full clinical examination by a veterinarian pre-study and prior to termination. The examination included cardiac and pulmonary auscultation and indirect ophthalmoscopy.

2. **Body weight:** The weight of each dog was recorded prior to dosing, weekly during dosing and at termination. Body weight gains were not reported.

3. **Food consumption:** Food consumption was determined daily for each animal beginning 2 weeks prior to dosing and continuing throughout the treatment period. Food consumption was reported weekly (g/dog/day).

4. **Ophthalmoscopic examination:** The eyes of all dogs were examined by a veterinarian pre-study and prior to termination using indirect ophthalmoscopy.

5. **Hematology and clinical chemistry:** Before feeding, jugular vein blood samples were collected during Weeks -1, 13, 26, and 52 of treatment. The following CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*	X	Abnormal morphology
	(Activated partial thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for chronic studies based on Guideline 870.4100.

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg. *)	X	Total bilirubin*
X	Alkaline phosphatase (ALP)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		Albumin/globulin ratio
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
X	Gamma-glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase*		

* Recommended for chronic studies based on Guideline 870.4100.

6. Urinalysis: Urine was collected by catheterization during Weeks -1, 13, 26, and 52. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood*
X	Sediment (microscopic)		Nitrate
X	Protein*		Urobilinogen

* Recommended for chronic studies based on Guideline 870.4100.

7. Sacrifice and pathology: At study termination, all animals were sacrificed by exsanguination under terminal anesthesia induced by intravenous injection of sodium pentobarbitone. All animals were subjected to a gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs were weighed, and bilateral organs were weighed separately.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta, abdominal*	XX	Brain (multiple sections)*+
X	Salivary glands*	X	Heart*+	X	Peripheral nerve* (sciatic)
X	Esophagus*	X	Bone marrow*	X	Spinal cord*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	XX	Parathyroids* [a]
X	Rectum*	X	Urinary bladder*	XX	Thyroids* [a]
XX	Liver*+	XX	Testes*+		OTHER
X	Gall bladder*	X	Epididymides*+	X	Bone (sternum and femur)
X	Pancreas*	X	Prostate*	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries*+	X	Skin*
X	Trachea*	X	Uterus*+	X	Stifle joint
X	Lung*++	X	Mammary gland* (females)	X	All gross lesions and masses*
	Nose*	X	Vagina (with cervix)		
	Pharynx*	X	Oviducts		
	Larynx*				

a The parathyroids were weighed with the thyroid.
* Required for chronic studies based on Guideline 870.4100.
\+ Organ weight required in chronic studies.
++ Organ weight required if inhalation route.

All tissues were submitted for histology except the femur which was stored. The Sponsor stated that the tissues were fixed in appropriate fixative. Tissue samples were processed routinely and stained with hematoxylin and eosin.

II. RESULTS

A OBSERVATIONS

1. Mortality: All animals were euthanized on schedule.

2. Clinical signs of toxicity: The 500 ppm females were considered thin (3/4 dogs, 62 total observations) compared to controls (1/4 dogs, 11 total observations). This observation was corroborated by body weight measurements. Reddening of the gums was noted in the 500 ppm males (3/4 dogs, 24 total observations) compared to 0 in controls and other groups. Fluid feces were observed more frequently in the 500 ppm males (2/4 dogs, 24 total observations) than in the controls (2/4 dogs, 3 total observations). The toxicological significance of the red gums and fluid feces was equivocal.

B. BODY WEIGHT AND WEIGHT GAIN: Body weight and body weight gain data are presented in Table 2. Body weights were decreased ($p \leq 0.05$) by 5-11% in the 500 ppm males during Weeks 2-30. A minor increase of 2% was noted in the 150 ppm males during Week 2. Body weights were decreased ($p \leq 0.05$) by 3-15% in the 500 ppm females throughout the study, with the exception of the final week. At 500 ppm, males lost weight

during the first 2 weeks, and females lost weight during the first week. All other body weight values in the treated groups were statistically similar to the controls. Body weight gains for the interval of Weeks 1-13 were decreased by 75-77% in the 500 ppm group. At 50 and 150 ppm, overall (Weeks 1-53) body weight gains were increased by 20-21% in the males and 26-35% in the females. At 500 ppm, overall body weight gains were decreased by 27% in the males and 38% in the females.

TABLE 2: Mean body weights (BW) and body weight gains (BWG) in kg [a]

Parameter/interval	0 ppm	50 ppm	150 ppm	500 ppm
Males				
BW Wk 1 [b]	9.15±1.48	9.25±0.99	8.98±1.10	8.95±0.74
BW Wk 2 [c]	9.14	9.15	9.29* (↑2)	8.72** (↓5)
BW Wk 17 [c]	10.70	10.68	10.82	9.57** (↓11)
BW Wk 53 [c]	11.21	11.67	11.59	10.56
BWG Wk 1-13 [d]	1.40	1.38	1.50	0.35 (↓75)
BWG Wk 13-26	0.43	0.62	0.47	0.28 (↓35)
BWG Wk 26-53	0.27	0.53	0.58	0.90 (↑233)
Overall BWG Wk 1-53	2.10	2.53 (↑20)	2.55 (↑21)	1.53 (↓27)
Females				
BW Wk 1 [b]	7.75±0.47	7.88±0.59	7.85±0.84	7.88±0.49
BW Wk 2 [c]	7.88	8.04	7.91	7.61* (↓3)
BW Wk 36 [c]	9.84	10.11	10.04	8.38** (↓15)
BW Wk 53 [c]	9.91	10.30	10.50	9.02
BWG Wk 1-13 [d]	1.20	1.35	1.25	0.27 (↓77)
BWG Wk 13-26	0.38	0.65	0.83	0.13 (↓66)
BWG Wk 26-53	0.40	0.50	0.60	0.82 (↑105)
Overall BWG Wk 1-53	1.98	2.50 (↑26)	2.68 (↑35)	1.22 (↓38)

a Data (n=4) were obtained from Table 8 on pages 56-69 in MRID 48073741. Percent difference from controls is included in parentheses, and was calculated by the reviewers.
b Mean weights ± SD
c Adjusted (covariable is Week 1) mean weights
d Body weight gains were calculated by reviewers from mean body weights reported in the cited data. Statistical analyses were not performed.
* Significantly different ($p \leq 0.05$) from the control groups
** Significantly different ($p \leq 0.01$) from the control groups

C. <u>FOOD CONSUMPTION</u>: Generally throughout the study at 500 ppm, food consumption decreased ($p \leq 0.05$) by 5-34% in males and by 6-36% in females (Table 3). Food consumption was similar in the other dose groups to controls.

TABLE 3. Mean (±SD) food consumption (g) in dogs treated with Picoxystrobin in the diet for 52 weeks [a]				
Week	Dose (ppm)			
	0	50	150	500
Males				
1	340±20	350±0	349±2	226±57** (↓34)
7	346±8	350±0	347±7	328±21* (↓5)
52	349±3	350±0	340±20	331±26 (↓5)
Females				
1	296±5	300±0	288±24	188±29** (↓36)
10	300±0	300±0	290±20	283±6* (↓6)
52	282±35	300±0	298±5	295±9 (↑5)

a Data (n=4) were obtained from Table 9 on pages 70-81 in MRID 48073741. Percent difference from controls is included in parentheses, and was calculated by the reviewers.
* Significantly different ($p \leq 0.05$) from the control groups
** Significantly different ($p \leq 0.01$) from the control groups

D. OPHTHALMOSCOPIC EXAMINATION: No eye abnormalities were detected.

E. BLOOD ANALYSES

1. **Hematology:** No adverse, treatment-related effects were observed on hematology parameters. The neutrophil count was decreased ($p \leq 0.05$) by 31% in the 500 ppm females; however, no other effects on total leukocyte count or lymphocyte, monocyte, eosinophil, and basophil counts were observed. Therefore, this slight decrease was considered incidental. Several differences ($p \leq 0.05$) were noted, but were minor, transient, and/or unrelated to dose.

2. **Clinical chemistry:** No adverse, treatment-related effects were observed on clinical chemistry parameters. Several differences ($p \leq 0.05$) were noted, but were minor, transient, and/or unrelated to dose.

F. URINALYSIS: No treatment-related effects were noted during urinalysis.

G. SACRIFICE AND PATHOLOGY

1. **Organ weight:** No adverse, treatment-related effect was observed on organ weights. Adjusted (terminal weight covariable) thyroid weights were increased ($p \leq 0.05$) by 21%, but no corroborating evidence of a pathological effect was found in the thyroid.

2. **Gross pathology:** No treatment-related effect was observed during necropsy.

3. **Microscopic pathology:** Histopathology results did not show any treatment-related effect.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The LOAEL was 500 ppm, based upon reduced growth and food consumption in both sexes. The NOAEL was 150 ppm.

B. REVIEWER COMMENTS: No adverse, treatment-related effects were observed on mortality, ophthalmology, hematology, clinical chemistry, urinalysis, organ weights, or gross or histological pathology.

The 500 ppm females were considered thin (3/4 dogs, 62 total observations) compared to controls (1/4 dog, 11 total observations). This observation was corroborated by reduced body weights.

At 500 ppm, body weights were decreased ($p \leq 0.05$) by 5-11% in the males during Weeks 2-30. Body weights were decreased ($p \leq 0.05$) by 3-15% in the females throughout the study, with the exception of the final week. Males lost weight during the first 2 weeks, and females lost weight during the first week. Body weight gains for the interval of Weeks 1-13 were decreased by 75-77% in both sexes, contributing to overall (Weeks 1-53) decreased body weight gains of 27% in the males and 38% in the females. Generally throughout the study, food consumption decreased ($p \leq 0.05$) by 5-34% in males and by 6-36% in females.

Reddening of the gums was noted in the 500 ppm males (3/4 dogs, 24 total observations) compared to 0 in controls and other groups. Fluid feces were observed more frequently in the 500 ppm males (2/4 dogs, 24 total observations) than in the controls (2/4 dogs, 3 total observations). The toxicological significance of the red gums and fluid feces was equivocal.

At 50 and 150 ppm, body weight gains were increased by 20-21% in the males and 26-35% in the females. Increased body weight gains are typically not considered adverse.

The LOAEL is 500 ppm (equivalent to 16.1/15.7 mg/kg/day in males/females), due to decreased body weights, body weight gains, and food consumption in both sexes. The NOAEL is 150 ppm (equivalent to 4.8/4.6 mg/kg/day in males/females).

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.4100b, OECD 452) for a chronic oral toxicity study in dogs.

C. STUDY DEFICIENCIES: The following deficiencies were noted but do not change the conclusions of the reviewer:

- No blood clotting measurements were performed.
- Nose, pharynx, and larynx tissues were not evaluated microscopically.
- Heart, spleen, epididymis, and uterus were not weighed.